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Optimal Fluid Use of Hypotensive Resuscitation and Transport

Timothy A. Pritts, MD

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Air Force Research Laboratory
711th Human Performance Wing
U.S. Air Force School of Aerospace Medicine
Aeromedical Research Department
2510 Fifth St., Bldg. 840
Wright-Patterson AFB, OH 45433-7913

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COL NICOLE ARMITAGE	DR. RICHARD A. HERSACK
Chief, En Route Care Research Division	Chair, Aeromedical Research Department

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14. ABSTRACT
Hypertonic crystalloid solutions, colloids, and fresh whole blood have all been proposed for prehospital resuscitation after hemorrhage. However, there are no direct comparisons of the efficacy of these different fluids. We compared Hextend, 3% hypertonic saline (HS), and fresh whole blood (FWB) in a porcine model of hemorrhagic shock. Female swine (n=5 per group) underwent splenectomy and pressure-controlled hemorrhage followed by resuscitation with Hextend, 3% HS, or FWB. They were maintained at a target mean arterial pressure (MAP) for 4 hours, holding or infusing fluid as necessary. Sham animals for comparison underwent splenectomy alone. The mean volume required to maintain target MAP was significantly higher for 3% HS (1016±386 mL) than for Hextend (346±299 mL, p=0.01) or FWB (467±189 mL, p=0.04). After 4 hours of resuscitation, the MAP in the 3% HS group (44±3 mmHg) was significantly lower than shams (56±7 mmHg, p=0.01). Three percent HS recipients had significantly worse metabolic acidosis and anemia than all other groups as well as significantly higher serum sodium than all other groups and significantly higher serum chloride than shams or FWB recipients. Serum interleukin-6 was significantly elevated in 3% HS recipients relative to Hextend recipients (105.3±58.6 vs. 38.6±27.1 pcg/mL, p=0.04). Hypertonic saline performed inferiorly to Hextend as a volume-expanding resuscitative fluid after hemorrhage. Based on our data, we would not recommend the use of 3% saline as the sole resuscitation fluid after hemorrhage.

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TABLE OF CONTENTS

Section **Page**

LIST OF FIGURES

Page

LIST OF TABLES

Page

1.0 SUMMARY

Hypertonic crystalloid solutions, colloids, and fresh whole blood have all been proposed for prehospital resuscitation after hemorrhage. However, there are no direct comparisons of the efficacy of these different fluids. We compared Hextend, 3% hypertonic saline (HS), and fresh whole blood (FWB) in a porcine model of hemorrhagic shock. Female swine (n=5 per group) underwent splenectomy and pressure-controlled hemorrhage followed by resuscitation with Hextend, 3% HS, or FWB. They were maintained at a target mean arterial pressure (MAP) for 4 hours, holding or infusing fluid as necessary. Sham animals for comparison underwent splenectomy alone. The mean volume required to maintain target MAP was significantly higher for 3% HS (1016±386 mL) than for Hextend (346±299 mL, p=0.01) or FWB (467±189 mL, p=0.04). After 4 hours of resuscitation, the MAP in the 3% HS group (44±3 mmHg) was significantly lower than shams (56±7 mmHg, p=0.01). Three percent HS recipients had significantly worse metabolic acidosis and anemia than all other groups as well as significantly higher serum sodium than all other groups and significantly higher serum chloride than shams or FWB recipients. Serum interleukin-6 was significantly elevated in 3% HS recipients relative to Hextend recipients (105.3±58.6 vs. 38.6±27.1 pcg/mL, p=0.04). Hypertonic saline performed inferiorly to Hextend as a volume-expanding resuscitative fluid after hemorrhage. Based on our data, we would not recommend the use of 3% saline as the sole resuscitation fluid after hemorrhage.

2.0 BACKGROUND

Hemorrhage remains the most common cause of potentially survivable mortality on the battlefield [1]. Current tactical combat casualty care resuscitation strategies use hypotensive resuscitation with Hextend (6% hetastarch in Ringer's lactate) to initially treat hemorrhagic shock and recommend a normotensive resuscitation with hypertonic (3%) saline to treat traumatic brain injury (TBI) patients. As the concept of tactical critical care evacuation teams evolves, supplemental resuscitation strategies may prove to be invaluable additions to the initial resuscitation fluid. These adjunctive measures could include the use of dried plasma for volume expansion and to mitigate coagulopathy.

Current use of Hextend as an initial volume expander in hemorrhagic shock is based on expert opinion and the result of previous Department of Defense consensus conferences. Recent clinical evidence shows increased mortality and organ injury with use of starch-containing solutions for resuscitation in critically ill patients [2-4]. Large animal experiments suggest that Hextend use after hemorrhage leads to increased coagulopathy compared to crystalloid resuscitation [5]. Hypertonic saline is a valuable resuscitation fluid following TBI and is already adopted by military medical teams for this injury pattern; however, the use of hypertonic saline for fluid management in the hypotensive resuscitation strategy following hemorrhage has not been tested. In a combined model of TBI and hemorrhagic shock, hypertonic saline was superior to Hextend as a resuscitation fluid [6]. We hypothesized that hypertonic saline would prove superior to Hextend when used in a hypotensive resuscitation strategy. If this hypothesis was proven true, this would allow far-forward medics and tactical critical care evacuation teams to carry and use a single fluid to treat the most common patterns of injury encountered on the battlefield.

The use of dried plasma is also supported in early resuscitation following traumatic hemorrhage and provides an alternative to scarce liquid blood resources in the far-forward setting. The use of dried plasma and standard resuscitation fluids has not been studied.

Our project answers two previously unanswered questions:

1. In a hypotensive resuscitation strategy, does hypertonic saline perform as well as Hextend in treatment of hemorrhagic shock?
2. In a model of complex liver injury, does dried plasma perform as well as Hextend in the treatment of hemorrhagic shock?

These experiments utilized a porcine model of hemorrhage and trauma/hemorrhage to test these hypotheses. By answering these questions, we hope to provide guidelines for optimal resuscitation to target potentially survivable deaths due to hemorrhage on the battlefield.

3.0 METHODS

3.1 Animal Housing and Preparation

All animal protocols were in accordance with the National Institutes of Health guidelines and were approved by the University of Cincinnati Institutional Animal Care and Use Committee. Twenty female Yorkshire pigs, mean weight 39.6±2.5 kg, were obtained from a local vendor (Isler Genetics, Prospect, OH) and allowed to acclimate in our facility for at least 48 hours. Animals were housed 1-2 per cage on standard bedding with food and water ad libitum. Food was withheld overnight the night before the procedure but water was not. All experiments were performed in the University of Cincinnati Center for Surgical Innovation surgical suite, Cincinnati, OH, beginning between 7 and 9 a.m. On the day of the procedure, animals were sedated with intramuscular 5 mg/kg telazol and 1 mg/kg xylazine (Henry Schein Animal Health, Dublin, OH), orotracheally intubated, positioned supine, and mechanically ventilated with a standard anesthesia ventilator (Ohmeda 7000, Ohmeda, Inc., Madison, WI) using standardized ventilator settings (inspired fraction of oxygen 1.0, tidal volume 10 mL/kg, positive end-expiratory pressure 5 cm H_2O, respiratory rate adjusted to achieve a target end-tidal carbon dioxide (CO_2) tension of 35±5 mmHg). We kept the inspired fraction of oxygen constant at 1.0 for all animals throughout the study in an effort to maintain consistency between animals and to avoid hypoxia during intermittent periods where pulse oximetry readings were lost secondary to hypotension. Anesthesia was maintained with inhaled isoflurane (Henry Schein Animal Health, Dublin, OH) for the duration of the study.

3.2 Instrumentation, Injury, Hemodynamic Monitoring, and Laboratory Values

Animals underwent cannulation of the following vessels: right femoral artery with a 20-gauge catheter (Teleflex Inc., Research Triangle Park, NC) for continuous blood pressure (BP) monitoring, right carotid artery with an 8.5 French catheter (Teleflex Inc., Research Triangle Park, NC) for hemorrhage and blood sampling, and right external jugular vein with a 7.5 French pulmonary artery catheter (Edwards Lifesciences, Irvine, CA) for measurement of pulmonary capillary wedge pressure (PCWP), central venous pressure (CVP), cardiac index, mean pulmonary artery (PA) pressure, and systemic vascular resistance (SVR, calculated).

Following placement of all monitoring devices, a splenectomy was performed via midline laparotomy, the spleen was weighed and discarded, and the laparotomy was closed. This was considered the baseline time point, followed by a 30-minute period for active hemorrhage and a 15-minute passive equilibration interval (collectively referred to as the "shock phase"), and a 4-hour resuscitation phase. At the end of the resuscitation phase, animals were euthanized. All animals received a continuous infusion of lactated Ringer's solution at 25 mL/h for the duration of the study to maintain catheter patency. Standard vital signs – heart rate (HR), rectal temperature (Temp), mean arterial pressure (MAP), and peripheral oxygen saturation by pulse oximetry – were recorded every 15 minutes. Pulmonary artery catheter measurements – PCWP, CVP, cardiac index, mean PA pressure, and SVR – were recorded at baseline, at the end of the shock phase, and then every hour during the resuscitation phase. Arterial blood gases, arterial lactate, and acid-base status – pH, base excess, serum bicarbonate (HCO_3), and arterial oxygen saturation (SpO_2) – were measured with a VetScan i-STAT point-of-care analyzer (Abaxis, Union City, CA) at baseline, at the end of the shock phase, and then every hour during the resuscitation phase. Hemoglobin, serum sodium, and serum chloride, were measured with i-STAT at baseline and immediately prior to euthanasia. Additional blood was collected at the end of the resuscitation phase, and the serum was isolated and assayed for the cytokines tumor necrosis factor alpha (TNFα), interleukin (IL) 6 (R&D Systems, Minneapolis, MN), and IL-10 (Life Technologies, Carlsbad, CA).

3.3 Hemorrhage and Resuscitation

Experiments for the hemorrhage model were carried out as detailed in a publication resulting from this work [7].

Sham animals (n=5) were not subjected to any further intervention after splenectomy and were maintained under general anesthesia for 4.75 hours, corresponding to the 45-minute shock phase and the 4-hour resuscitation phase of shocked animals. At the end of the resuscitation phase, final labs were analyzed and 1.5 liters of whole blood were removed via a sterile, citrated circuit. The whole blood was mixed with a citrate/phosphate/dextrose anticoagulant and stored for less than 48 hours at 4°C prior to use, in accordance with U.S. Department of Defense recommendations for whole blood transfusion [8]. This blood was subsequently used to resuscitate animals in the fresh whole blood (FWB) group. Due to infrastructure limitations and the need for this directed transfusion, a true randomization of animals to their respective groups was not possible. The experiments were performed in several rounds over the course of several months with animals for each of the four groups included in each round. On the day of use, individual animals were selected, anesthetized, and intubated by personnel without any knowledge of the experimental group assignments for these animals.

Pigs in each of the three shock groups (n=5 each) underwent a pressure-controlled hemorrhage by removing blood at 100 mL/min (Masterflex L/S pump, Cole Parmer, Vernon Hills, IL) to a MAP of 30±5 mmHg. This hypotensive state was actively maintained for 30 minutes by withdrawing blood as needed to keep the MAP within the target range, followed by a passive 15-minute equilibration period. At the end of the shock phase, the volume of shed blood was recorded, and resuscitation was initiated with Hextend, 3% hypertonic saline (HS), or FWB (Hextend and HS from Moore Medical, Farmington, CT). The infusion was stopped when the MAP reached 50 mmHg and then restarted as needed to maintain a MAP of 45-50 mmHg. The total volume infused over 4 hours was measured and recorded.

3.4 Trauma, Hemorrhage, and Resuscitation

After induction of anesthesia, pigs underwent laparotomy and splenectomy as described above. Pigs then were subjected to a Grade V liver injury using a standardized clamp system as previously described [9]. Pigs were then resuscitated with either Hextend or reconstituted lyophilized plasma [10,11]. Vital signs and arterial blood gasses were obtained at intervals.

3.5 Statistical Analysis

SigmaPlot version 11.0 was utilized for statistical comparisons (Systat Software, San Jose, CA). All data are presented as mean ± standard deviation (SD). Statistical comparisons between groups were made by Kruskal-Wallis test with Dunn's test for post hoc pairwise comparisons where appropriate. Significance was defined prior to analysis as $p<0.05$. The data were analyzed in a single batch after the completion of all experimental groups.

4.0 RESULTS

4.1 Hemorrhage Experiments

At baseline, there were no significant differences between any of the groups in animal weight, spleen weight, vital signs, PA catheter parameters, or laboratory values. There were also no differences between any of the shocked groups in shed blood volume or post-shock vital signs, hemodynamic parameters, or laboratory values, and all animals survived to the end of the observation period. The mean volume of shed blood for all shocked animals was 827±222 mL, corresponding to 29±7% of total blood volume. This led to a decrease in MAP from 68.9±9.7 mmHg at baseline to 34.7±4.1 mmHg at the end of the shock phase and an increase in HR from 88±9 bpm at baseline to 110±38 bpm at the end of the shock phase, corresponding to class III hemorrhagic shock. Animals resuscitated with 3% HS required the highest volumes to maintain the target MAP (1016±386 mL). This was significantly higher than Hextend (346±299 mL) but not significantly different from FWB (467±189 mL, Figure 1). The mean baseline MAP for all animals was 70±9 mmHg. There was a gradual decline in the MAP of sham animals over the course of the study to a final MAP of 56±7 mmHg. All shocked animals experienced an increase in MAP with resuscitation. However, at the end of the resuscitation phase, the MAP of animals resuscitated with 3% HS (44±3 mmHg) was significantly lower than that of shams, while the MAP of animals receiving Hextend or FWB was no different than shams (52±7 and 49±3 mmHg, respectively, Figure 2). There were no statistically significant differences between the three resuscitated groups.

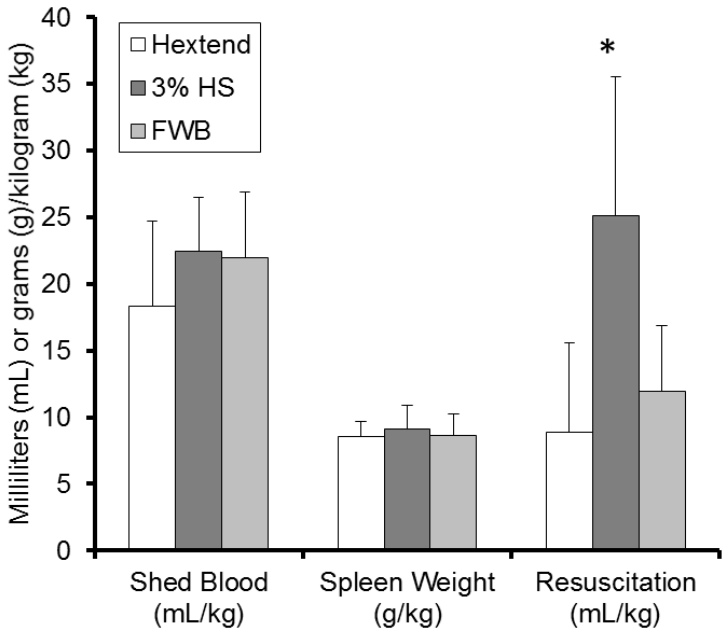

Figure 1. Shed blood volume, spleen weight, and resuscitation requirements in pigs. *There was no difference between groups in shed blood volume or spleen weight. However, animals resuscitated with 3% HS required significantly larger volumes than those receiving Hextend to achieve and maintain target BP. *p<0.05 vs. Hextend.*

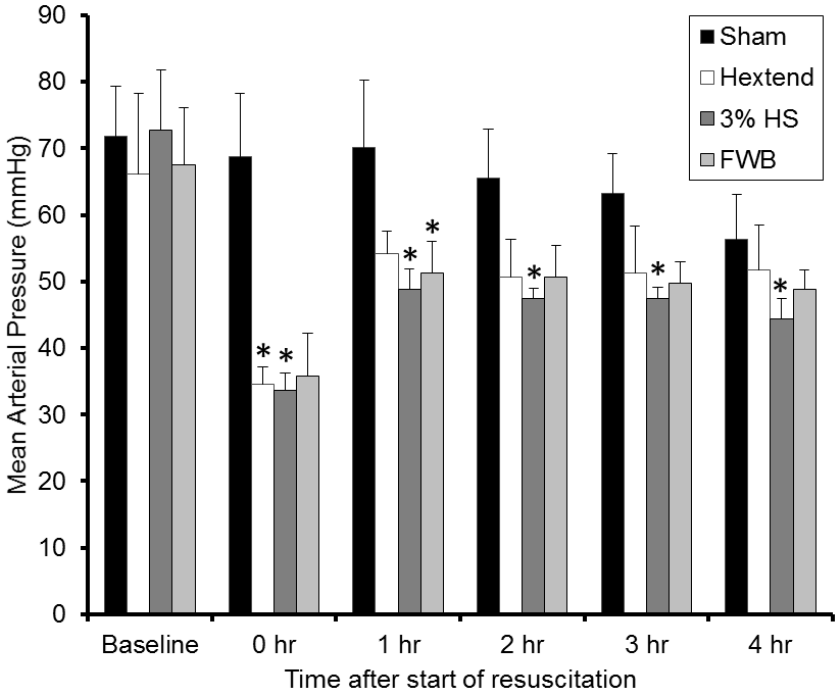

Figure 2. Mean arterial blood pressure after 4 h of resuscitation. *MAP is significantly lower than sham animals after 4 h of resuscitation with 3% HS. However, there is no significant difference from shams after 4 h of resuscitation with Hextend or FWB. *p<0.05 vs. sham.*

4.1.1 Pulmonary Artery Catheter Measurements. There were no significant differences in PCWP, CVP, cardiac index, or mean PA pressure between any of the three treatment groups at baseline, after shock, or during resuscitation (Table 1). All shocked groups experienced a significant decrease in cardiac output after shock with an overall post-shock cardiac index that was 52±18% of baseline. After 4 hours of resuscitation, the cardiac index values did not significantly differ between any of the groups. However, when the relative increase from the post-shock level was analyzed, animals resuscitated with 3% HS had the largest increase in cardiac index (242±49% of post-shock value after 4 hours, $p<0.05$ vs. sham), while Hextend and FWB led to no significant increase vs. shams (164±18% and 145±37% vs. 110±22% of post-shock value). Animals resuscitated with 3% HS had the lowest SVR after 4 hours of resuscitation (813±192 dyn*s/cm^5), significantly lower than Hextend recipients (1652±568 dyn*s/cm^5) but not significantly different from shams or FWB recipients (1067±66 and 1270±195 dyn*s/cm^5, respectively).

4.1.2 Laboratory Values. At baseline, there were no significant differences between any of the groups in any of the measured laboratory values. There were no significant differences between groups for arterial blood gases or lactate at any of the time points in the study. However, significant changes in acid-base status began to occur as early as 1 hour after the start of resuscitation and persisted through the end of the study (all values are listed in Table 2). After 1 hour of resuscitation, animals receiving 3% HS had significantly lower values than shams for pH and base excess. After 4 hours of resuscitation, 3% HS animals had significantly lower values than shams and FWB recipients for base excess and HCO_3 and significantly lower pH than FWB recipients. Hextend recipients were not significantly different from any of the other groups. After 4 hours of resuscitation, animals receiving 3% HS had the lowest hemoglobin level (5.3±0.7 g/dL), significantly lower than shams and FWB recipients (9.7±0.8 g/dL and 8.9±0.8 g/dL, respectively) but not significantly different from Hextend recipients (7.4±1.4 g/dL, Figure 3). At the end of the resuscitation, animals receiving 3% HS had higher serum sodium than shams (152.8±6.4 vs. 136±2.1 mEq/L, respectively) or FWB recipients (135.4±2.9 mEq/L), but did not significantly differ from Hextend recipients (135.4±2.7 mEq/L). Animals resuscitated with 3% HS also had higher serum chloride than FWB recipients (121.2±6.6 vs. 100.2±1.1 mEq/L, respectively) but were not significantly different from shams or Hextend recipients (101.4±2.3 and 102.4±2.9 mEq/L, respectively, Figure 4).

4.1.3 Cytokine Analysis. Serum IL-6 was significantly elevated in animals resuscitated with 3% HS and FWB relative to those resuscitated with Hextend (105.3±58.6 and 97.2±21 vs. 38.6±27.1 pcg/mL, respectively). There were no significant differences between any of the groups in serum levels of TNFα or IL-10 (Figure 5).

4.2 Trauma and Hemorrhage Experiments

After laparotomy and prior to hemorrhage, all pigs exhibited similar baseline characteristics for hemodynamic parameters (Table 3) and arterial blood gas analysis (Table 4). Creation of a complex liver injury resulted in immediate blood loss ranging from 220 mL to 1090 mL (mean + SD of 466.7 mL + 285.4 mL) as well as decreased MAP, systolic blood pressure (SBP), and diastolic blood pressure (DBP) as compared to control pigs (Table 3). Two pigs treated with plasma died within 20 minutes of initiation of resuscitation. This mortality rate

limited further analysis. No significant differences were seen in arterial blood gas analysis throughout the study period.

Table 1. Pulmonary Artery Catheter Measurements for Different Resuscitation Groups

Measurement	Sham Mean (SD)	Hextend Mean (SD)	3% HS Mean (SD)	FWB Mean (SD)
PCWP (mmHg)				
Baseline	11.8 (0.8)	10.8 (1.9)	11.2 (2.3)	11.6 (0.9)
Post-shock	12.0 (0)	9.0 (1.6)	8.0 (1.7)	8.4 (1.7)
1 h	11.2 (1.1)	10.0 (2.9)	9.4 (1.5)	10.2 (1.1)
2 h	10.8 (0.4)	9.4 (2.5)	9.2 (1.9)	10.4 (0.5)
3 h	11.0 (0.7)	9.6 (3.1)	10.2 (1.8)	11.2 (1.6)
4 h	11.2 (0.8)	9.0 (2.5)	10.0 (1.7)	10.8 (1.0)
CVP (mmHg)				
Baseline	10.0 (3.5)	9.6 (2.5)	10.2 (1.3)	9.8 (1.3)
Post-shock	9.4 (3.8)	7.6 (1.9)	8.6 (1.1)	6.8 (1.8)
1 h	10.0 (1.9)	8.4 (2.3)	10.4 (3.8)	8.0 (1.6)
2 h	9.6 (2.2)	8.4 (2.1)	10.8 (4.3)	8.8 (1.1)
3 h	9.6 (1.8)	8.6 (2.7)	11.2 (3.3)	9.2 (1.8)
4 h	10.0 (1.9)	8.4 (2.9)	10.8 (4.1)	9.6 (1.3)
Cardiac Index (L/min/m^2)				
Baseline	3.8 (0.7)	3.3 (0.9)	4.2 (0.6)	4.1 (0.4)
Post-shock	3.9 (0.5)	1.7 (0.3)[a]	1.8 (0.7)[a]	2.3 (0.7)
1 h	4.6 (0.4)	2.8 (0.5)[a]	3.4 (0.4)	3.7 (0.4)
2 h	4.4 (0.6)	2.7 (0.5)[a]	3.5 (0.8)	3.3 (0.6)
3 h	4.4 (0.7)	2.8 (0.7)[a]	3.7 (1.3)	3.3 (0.6)
4 h	4.2 (0.5)	2.8 (0.8)	4.2 (1.4)	3.1 (0.5)
Mean PA Pressure (mmHg)				
Baseline	18.2 (2.9)	20.4 (3.9)	19.6 (1.8)	18.6 (3.4)
Post-shock	20.2 (3.3)	15.2 (1.3)	15.4 (1.9)	17.0 (2.8)
1 h	20.0 (2.1)	17.2 (1.8)	20.2 (4.5)	19.4 (2.9)
2 h	20.4 (1.7)	17.8 (3.4)	19.6 (2.9)	19.2 (2.8)
3 h	20.2 (1.9)	17.2 (2.4)	19.2 (2.4)	20.6 (2.1)
4 h	20.2 (2.2)	17.2 (2.7)	19.0 (2.5)	21.4 (1.8)
SVR (dyn*s/cm^5)				
Baseline	1589 (355)	1805 (405)	1431 (160)	1373 (114)
Post-shock	1479 (195)	1708 (658)	1457 (462)	1277 (141)
1 h	1273 (141)	1695 (408)	1085 (202)	1152 (182)
2 h	1229 (117)	1614 (410)	1037 (244)	1264 (260)
3 h	1190 (186)	1593 (391)	982 (238)[b]	1226 (179)
4 h	1067 (66)	1652 (568)	813 (192)[b]	1270 (195)

[a]$p<0.05$ vs. sham.
[b]$p<0.05$ vs. Hextend.

Table 2. Acid-Base Values for Different Resuscitation Groups

Acid-Base	Sham Mean (SD)	Hextend Mean (SD)	3% HS Mean (SD)	FWB Mean (SD)
pH				
Baseline	7.54 (0.03)	7.48 (0.06)	7.5 (0.03)	7.53 (0.03)
Post-shock	7.54 (0.02)	7.46 (0.06)	7.51 (0.02)	7.51 (0.06)
1 h	7.52 (0.01)	7.45 (0.06)	7.41 (0.06)[a]	7.48 (0.06)
2 h	7.52 (0.01)	7.47 (0.05)	7.43 (0.03)[a,b]	7.51 (0.04)
3 h	7.5 (0.02)	7.47 (0.05)	7.42 (0.04)[b]	7.52 (0.06)
4 h	7.49 (0.02)	7.46 (0.06)	7.41 (0.02)[b]	7.52 (0.07)
Base Excess (mEq/L)				
Baseline	12.8 (2.3)	9.6 (2.4)	11.4 (1.8)	11.4 (2.0)
Post-shock	13 (2.4)	5.8 (1.5)[a]	8.8 (2.8)	8.6 (4.2)
1 h	12.6 (1.7)	8.8 (1.9)	5.4 (4.2)[a]	9.6 (2.2)
2 h	13 (1.6)	9.2 (1.9)	5.6 (3.1)[a,b]	12 (1.6)
3 h	11.6 (1.8)	10 (1.2)	4.6 (1.5)[a,b]	13.0 (1.9)
4 h	10.8 (2.2)	9.6 (2.1)	3.6 (2.2)[a,b]	12.2 (2.7)
HCO_3 (mEq/L)				
Baseline	35.3 (2.3)	32.8 (2.3)	34.5 (1.8)	33.9 (1.7)
Post-shock	35.5 (2.3)	29.9 (1.7)[a]	31.8 (2.5)	31.6 (3.9)
1 h	35.5 (1.9)	32.6 (2.1)	30.1 (3.7)	33.3 (1.9)
2 h	35.7 (1.3)	32.9 (1.9)	29.8 (2.9)[a]	35.0 (1.0)
3 h	34.7 (1.7)	33.8 (1.7)	29.1 (2.1)[a,b]	35.6 (1.0)
4 h	35.8 (3.4)	33.5 (2.4)	28.3 (2.4)[a,b]	35.1 (1.9)

[a]p<0.05 vs. sham.
[b]p<0.05 vs. FWB.

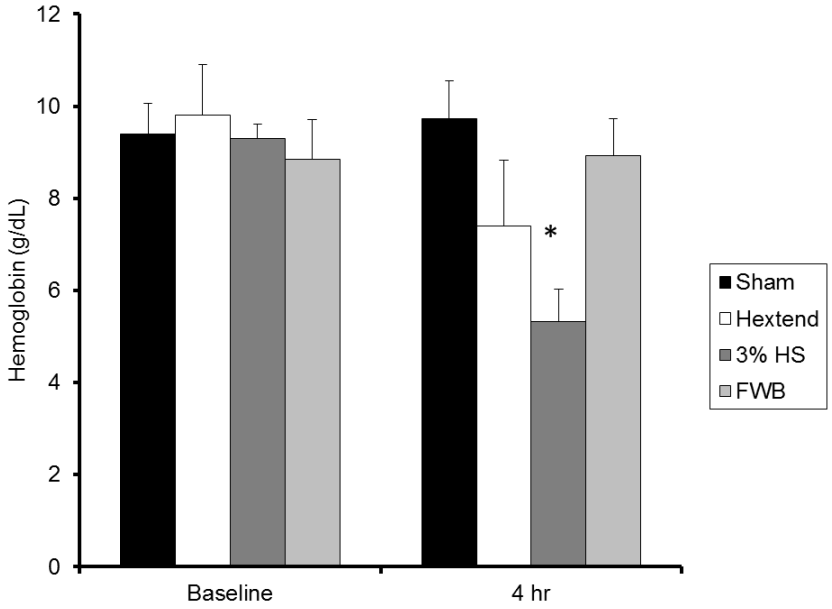

Figure 3. Hemoglobin measurements. *Animals resuscitated with 3% HS had significantly lower hemoglobin than shams or those resuscitated with FWB. *p<0.05 vs. sham and FWB.*

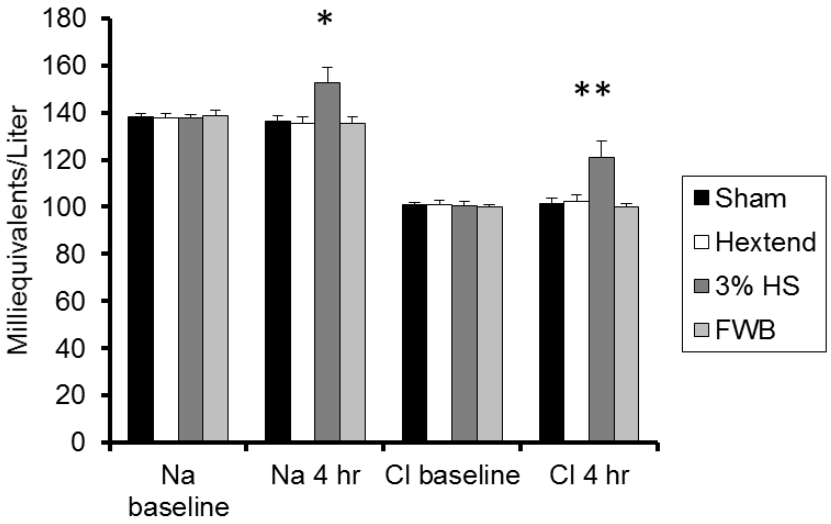

Figure 4. Serum sodium and chloride levels during resuscitation. *Animals resuscitated with 3% HS had significantly elevated levels of serum sodium (Na) relative to those resuscitated with Hextend or FWB and significantly elevated levels of serum chloride (Cl) relative to those resuscitated with FWB. *p<0.05 vs. Hextend and FWB. **p<0.05.*

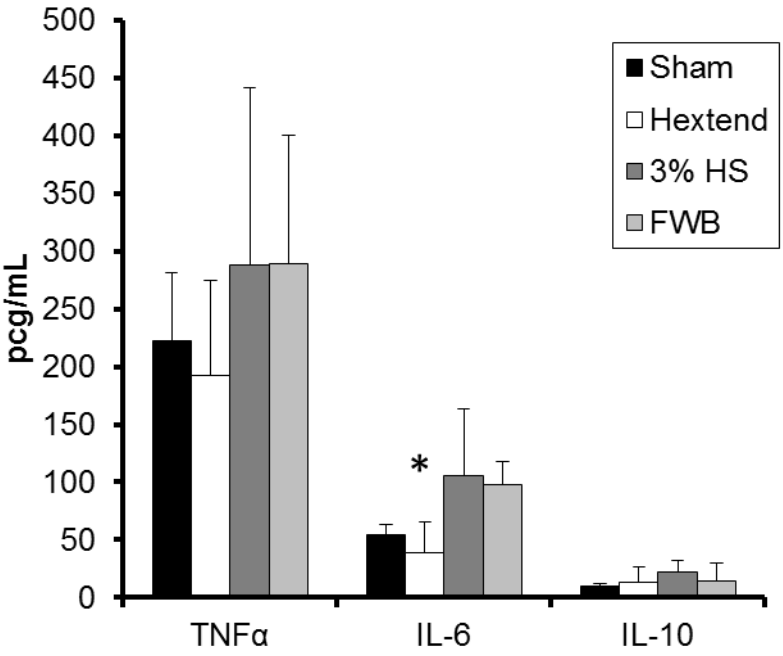

Figure 5. Serum cytokine levels. *Animals resuscitated with 3% HS or FWB had significantly higher serum levels of IL-6 than those resuscitated with Hextend. There was no significant difference between groups in TNFα or IL-10. *p<0.05 vs. 3% HS and FWB.*

Table 3. Hemodynamic Values After Trauma, Liver Injury, and Resuscitation

Group	Parameter	Baseline		10 min		20 min		30 min		40 min		50 min		1 h		2 h		3 h		4 h	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Control	HR	94.8	10.9	97.2	13.5	98.0	12.1	101.2	11.9	103.4	11.8	100.8	11.8	100.8	11.8	103.4	12.5	103.6	13.4	107.2	15.4
Hextend	HR	93.8	14.7	96.8	14.7	94.2	12.8	96.6	10.4	103.3	10.0	95.6	10.0	95.6	12.1	101.5	14.7	110.5	25.6	112.5	30.3
Plasma	HR	82.7	7.1	95.7	14.7	99.0	15.6	86.0	0.0	105.6	0.0	96.0	0.0	85.0	0.0	94.0	0.0	106.0	0.0	161.0	0.0
Control	BP-MAP	69.2	36.4	87.0	11.4	88.8	13.8	88.0	12.3	78.3	15.0	87.3	15.0	86.0	12.8	78.4	11.8	74.0	8.8	75.0	13.0
Hextend	BP-MAP	64.8	9.0	59.6	9.5	53.6	12.7	59.6	16.3	59.6	10.6	64.0	10.6	60.2	5.2	60.5	5.7	60.3	6.2	57.0	5.9
Plasma	BP-MAP	59.3	3.1	55.7	8.5	31.0	11.4	45.0	0.0	59.1	0.0	56.0	0.0	53.0	0.0	60.0	0.0	60.0	0.0	62.0	0.0
Control	SBP	82.0	43.1	104.8	12.5	105.0	18.2	102.0	12.8	94.6	11.9	102.3	11.9	102.5	14.0	96.0	13.0	92.6	13.7	91.8	15.1
Hextend	SBP	83.8	11.6	75.0	13.4	74.8	10.2	78.2	14.7	78.0	10.7	85.8	10.7	74.6	15.8	79.8	9.8	78.5	9.1	77.0	9.4
Plasma	SBP	76.7	4.2	51.7	18.4	39.0	15.6	59.0	0.0	72.9	0.0	70.0	0.0	68.0	0.0	74.0	0.0	73.0	0.0	71.0	0.0
Control	DBP	59.0	31.5	74.0	11.5	76.0	13.6	77.8	11.0	67.6	15.6	75.3	15.6	73.8	13.3	65.6	12.6	64.8	10.8	65.4	15.0
Hextend	DBP	54.0	7.2	49.2	8.8	51.6	14.3	49.6	13.7	46.6	5.2	49.2	5.2	45.2	8.7	47.5	2.6	46.8	3.6	44.3	5.7
Plasma	DBP	50.7	2.5	34.7	11.1	25.3	10.4	39.0	0.0	47.8	0.0	47.0	0.0	44.0	0.0	49.0	0.0	48.0	0.0	44.0	0.0
Control	CO_2	37.6	0.5	38.0	0.9	38.0	0.7	37.8	1.4	37.6	1.4	37.8	1.4	39.0	1.5	37.2	1.2	37.6	1.0	38.0	0.9
Hextend	CO_2	38.0	1.9	35.6	0.5	36.2	2.0	37.2	2.1	36.5	2.5	37.4	2.5	35.8	2.5	36.8	1.5	37.0	1.9	36.5	2.3
Plasma	CO_2	35.7	0.6	27.7	8.5	20.7	9.0	37.0	0.0	36.6	0.0	37.0	0.0	35.0	0.0	39.0	0.0	36.0	0.0	37.0	0.0
Control	SpO_2	98.6	1.5	98.0	2.5	98.6	1.4	98.4	1.4	98.7	1.4	98.4	1.4	98.4	1.5	98.8	1.6	98.8	1.6	98.8	1.2
Hextend	SpO_2	97.8	2.2	98.8	1.3	94.4	6.3	92.6	6.1	95.3	1.9	96.6	1.9	94.2	4.8	96.0	2.4	95.5	2.9	93.5	5.1
Plasma	SpO_2	97.7	1.5	93.7	3.4	95.0	1.0	96.0	0.0	97.2	0.0	97.0	0.0	97.0	0.0	98.0	0.0	97.0	0.0	96.0	0.0
Control	Temp	99.0	1.4	98.9	1.5	98.9	1.4	98.9	1.4	99.4	1.5	98.9	1.5	99.0	1.4	99.3	1.5	99.7	1.5	100.1	1.5
Hextend	Temp	99.5	1.4	68.1	29.5	74.3	28.7	74.2	28.7	79.4	29.7	67.8	29.7	73.8	29.9	82.8	25.9	82.8	25.7	82.8	25.6
Plasma	Temp	97.9	0.9	77.5	28.7	77.4	28.6	98.2	0.0	96.8	0.0	98.2	0.0	98.0	0.0	96.8	0.0	96.3	0.0	95.6	0.0

Table 4. Arterial Blood Gas Values After Trauma, Liver Injury, and Resuscitation

Time Point	Group	pH	PCO_2	PO_2	BEecf	HCO_3	sO_2	Na	K	iCa	Glu	Hct	Hgb
Baseline	Control	7.5	38.5	382.0	9.8	32.5	100.0	138.5	3.6	1.3	45.8	23.0	7.8
	Hextend	7.5	44.8	494.2	10.4	34.0	100.0	138.0	4.4	1.3	58.0	22.8	7.8
	Plasma	7.5	42.8	487.0	9.7	33.0	100.0	136.3	4.1	1.3	54.0	26.3	8.9
1-h	Control	7.5	42.5	410.8	12.3	35.1	100.0	137.0	4.1	1.3	43.8	27.0	9.2
	Hextend	7.5	41.7	471.6	7.8	31.3	100.0	138.6	4.7	1.3	44.8	14.0	4.8
	Plasma	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
2-h	Control	7.5	42.1	357.0	11.0	34.0	98.8	135.8	4.7	1.3	45.3	27.3	9.3
	Hextend	7.5	45.5	454.5	11.3	34.5	100.0	137.5	5.1	1.3	40.3	19.3	6.6
	Plasma	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
3-h	Control	7.5	42.4	434.0	11.5	35.1	100.0	135.5	5.1	1.3	53.3	27.3	9.3
	Hextend	7.5	46.1	486.8	11.0	34.6	100.0	136.5	5.6	1.3	42.0	18.0	6.1
	Plasma	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
4-h	Control	7.5	42.7	420.8	11.3	34.3	100.0	135.5	5.3	1.3	61.5	27.0	9.2
	Hextend	7.5	46.5	491.0	9.5	33.4	100.0	136.5	5.8	1.3	47.0	18.0	6.1
	Plasma	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

Note: BEecf = base excess of extracellular fluid; Glu = glucose; Hgb = hemoglobin; Hct = hematocrit; iCa = ionized calcium; K = potassium; Na = sodium; ND = no data; PCO_2 = partial pressure of carbon dioxide; PO_2 = partial pressure of oxygen; sO_2 = oxygen saturation.

5.0 DISCUSSION

In the present series of experiments, we compared the efficacy of 3% HS, Hextend, and FWB in the resuscitation from hemorrhage in a porcine model of controlled hemorrhage. Based on the existing literature concerning resuscitation with 3% HS, we expected that it would perform comparably to Hextend. However, we found that significantly larger volumes of 3% HS were required to maintain the target BP. Further, even with larger volumes, 3% HS was inferior to Hextend at maintaining a target BP and was also associated with worsened acidosis, anemia, and electrolyte derangements relative to Hextend and FWB.

The rationale for our study design was that use of 3% HS for resuscitation from hemorrhage could lead to consolidation of resources, since 3% HS is already carried by far-forward military teams for its utility in managing intracranial hypertension following TBI. However, the primary failure of 3% HS that we observed in the present study was the inability to adequately maintain target hemodynamic parameters without ongoing infusion, such that significantly higher volumes of 3% HS were required relative to Hextend. We did observe a greater increase in cardiac index and a significantly lower SVR after resuscitation with 3% HS than was observed with Hextend or FWB, a finding consistent with previous studies [12]. However, this did not correlate with increased systemic BP or decreased acidosis. Han et al. have suggested that the ability of 3% HS to increase MAP may be maximized by increasing the rate of infusion, but they have not measured this effect beyond 1 hour [13]. In our study there was an initial increase in MAP with all three resuscitation fluids, but this effect was less durable with 3% HS than with the other fluids. Despite the limitations of a pressure-guided resuscitation, other measures are frequently unavailable in the far-forward setting, and the current Tactical Combat Casualty Care guidelines recommend continued resuscitation of soldiers in hemorrhagic shock "until a palpable radial pulse, improved mental status or systolic BP of 80-90 mmHg is present" [14]. Based on the existing literature and the results of the present study, we cannot currently

advocate the use of 3% HS as the sole or primary fluid in far-forward resuscitation where carrying capacity is limited and there may be significant delays to providing definitive care.

In contrast to 3% HS, we found that resuscitation with Hextend led to rapid restoration of systemic BP with infrequent requirements for further infusion, performing comparably to FWB in all hemodynamic and acid-base parameters. It is notable that resuscitation with Hextend was associated with significantly lower levels of circulating IL-6 relative to 3% HS. However, there was also a significant increase in IL-6 after resuscitation with FWB relative to Hextend, even though they performed comparably in other respects. It may be that cytokines were released from the nonleukocyte-reduced donor blood or that the transfusion stimulated a host response, but we were unable to further investigate this question with the design of the present study. Regardless, our findings suggest that Hextend causes less acute inflammation than 3% HS during initial volume expansion after hemorrhage.

Recent experiences with FWB transfusion in military operations suggest that collection and use are feasible and that there may be significant benefits to using FWB in combat casualties, but its role remains to be fully defined [15,16]. In many situations, collection and transfusion of FWB are impossible or impractical, and Hextend is currently the resuscitation fluid of choice for U.S. military teams without access to blood products. However, there have been recent concerns about the safety of hydroxyethyl starch solutions, primarily related to their association with increased renal injury in critically ill and septic patients [17]. We did not study end organ damage and would not expect to see significant differences in renal function this early in resuscitation, but this is certainly an important consideration in the overall resuscitation strategy and is a target of our ongoing research in this area.

Additional experiments attempted to compare the efficacy of plasma to Hextend in a porcine model of trauma, complex liver injury, and hemorrhage. Interpretation of data from these experiments is very limited due to immediate mortality of pigs in the plasma group. This may have been related to a transient increase in BP upon initiating the infusion with resultant loss of tamponade of the injury, but this observation is anecdotal and speculative.

There are several limitations of the present study, and our data should be interpreted with caution. First, we chose a controlled hemorrhage model for the initial experiments to facilitate direct comparisons of required resuscitation volumes for each of the resuscitation fluids. A model of uncontrolled hemorrhage or polytrauma could provide additional or different information, such as the hemostatic potential of different resuscitation fluids. Future studies should examine this aspect of resuscitation, particularly given concerns that hypertonic solutions may contribute to coagulopathy [18]. Second, we only used female animals in this study. This was to limit variability given the relatively small sample size in our experimental design. However, it should be noted that estrogens can be active in modulating the response to hemorrhage, and caution should be used in extrapolating these findings to other populations. Additionally, this study only focused on resuscitation in the acute post-injury phase; further study is needed to understand the longer term effects. We would particularly like to investigate subacute and long-term differences in survival, lung injury, immunomodulation, and renal function with these different resuscitation strategies in our large animal model. Nonetheless, the data garnered from this study provide a valuable starting point for the comparison of these different fluids and help guide future directions for this area of investigation.

6.0 CONCLUSIONS

This study provides important information to help define the roles of several modern tools for military teams treating acute hemorrhagic shock in the remote setting when blood is not available. Based on our data, we would recommend the use of Hextend, rather than 3% saline, to resuscitate after hemorrhage.

7.0 REFERENCES

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LIST OF ABBREVIATIONS AND ACRONYMS

BP	blood pressure
CVP	central venous pressure
DBP	diastolic blood pressure
FWB	fresh whole blood
HCO_3	bicarbonate
HR	heart rate
HS	hypertonic saline
IL	interleukin
MAP	mean arterial pressure
PA	pulmonary artery
PCWP	pulmonary capillary wedge pressure
SBP	systolic blood pressure
SD	standard deviation
SpO_2	arterial oxygen saturation
SVR	systemic vascular resistance
TBI	traumatic brain injury
Temp	temperature
TNFα	tumor necrosis factor